OBSERVATIONS ON THE LIFE HISTORY OF *TRYPANOSOMA LEWISI* IN THE RAT LOUSE *(HAEMATOPINUS SPINULOSUS)*

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For over a year we have carried out experiments concerning the mode of transmission of *Trypanosoma lewisi* by means of the rat louse *Haematopinus spinulosus.* Although our results are far from complete, circumstances compel us to abandon this research for the present.

We have succeeded in transmitting *T. lewisi* by means of *Haematopinus spinulosus*, and, with the aid of wet fixation methods, in modifying conclusions which have previously been drawn concerning the cytológical characters of this parasite in specimens stained by Romanowsky. We do not intend in the present paper to go into a detailed, theoretical discussion, which we reserve for a future communication, but only to bring forward some new facts and observations concerning the life-history of *T. lewisi* in its intermediate host.

Prowazek[1] was the first to draw attention to the life-history of *T. lewisi* in the rat louse. For some years Prowazek's conclusions were generally accepted, without being confirmed by other workers. Patton's and Strickland's[2] attempt to confirm Prowazek's work led them to the conclusion, that Prowazek had described part of the life cycle of a natural flagellate, *Crithidia* sp., of *Haematopinus spinulosus*, which they believe has no connection with *T. lewisi.*

Nuttall,[3] using thirty lice, was able to transmit, in the first of two experiments, *T. lewisi* from an infected to a normal animal. In the second experiment, ten lice were transferred from a wild to an uninfected rat without, however, giving rise to an infection in the latter. As he was unable to trace any development of *T. lewisi* in the rat louse, he seriously doubts whether any such development occurs.

Baldry,[4] on the other hand, working in Hartmann's laboratory was not only able to obtain positive results in his transmission experiments, but also fully confirms Prowazek's observations regarding conjugation. He asserts that the developmental cycle takes place within eight to ten days, and that it is a very small *Crithidia*-like form of *T. lewisi* in the louse which gives rise to the new infection.

TRANSMISSION EXPERIMENTS

In three of our experiments we have been able to transmit *T. lewisi* by means of the rat louse. We usually collected well-fed lice from a heavily infected rat and transferred them, after an interval of twenty hours, to a normal rat. In one experiment, twenty-four, and in the other two experiments, forty, lice were used. In the first two cases parasites appeared in the peripheral blood after an incubation period of eight days, and in the third after ten days. In each case the animal developed a typical infection.

In two further experiments ten lice were used after having been kept for two days in test tubes, but in neither did an infection result, although the animals were kept under observation for a considerable period.

Method. For the study of the parasite in the intermediate host, rat-lice were collected from an infected animal. The heads of the lice were snipped off with a small pair of scissors, and the gut contents teased out in a small amount of physiological saline solution. Usually a film was made of the gut contents of five to ten lice, and while still wet dipped into Flemming's or Hermann's solution, or sublimate-acetic-alcohol. Flemming's solution, however, gave the best and most satisfactory results, and parasites fixed in this way showed less deformity than by any other method. If sublimate-acetic-alcohol was used, the parasites always seemed to be somewhat shrunken.

The specimens were stained with Breinl's safranine-methylene-blue tannin orange method and Breinl's modification of Heidenhain, as described in a previous paper.[5] The comparative use of these two methods was of great service. In the former method, granules, which were often present in large numbers, stained brilliantly with safranine, at times masking the nucleus and blepharoplast and often rendering the interpretation of cytological details difficult. When, on the other hand, Heidenhain's haematoxylin method was used the granules remained unstained. In this method, however, it was more difficult to obtain as perfect a differentiation of the details of the nucleus as with safranine methylene blue. Fresh examination was also constantly practised, and sometimes a parasite was kept under observation for as long as twenty-four hours.

It seems surprising that Nuttall and his co-workers were unable to observe developmental changes in rat-lice taken from rats infected with *T. lewisi*. However, for nearly four months we saw nothing but degenerated trypanosomes in our rat-lice, so that we nearly abandoned further research. Ultimately, in one case developmental forms were found in lice taken from an infected animal, and it became apparent that during the first stages of infection, so long as dividing and segmenting forms were present in the blood, the trypanosomes taken up by the louse only degenerated. On the other hand, so soon as division forms no longer appeared in the peripheral blood, the trypanosomes taken up by the louse underwent marked changes in those cases in which the rat's blood was literally swarming with trypanosomes. If, however, there were only comparatively few trypanosomes in the blood (about seventy to eighty to a microscopic field), hardly any changes were noticeable in the gut contents of the louse.

Thus, although nothing but degenerated trypanosomes were present in the gut contents of lice taken from rats in the early stages of infection, nevertheless, developmental forms made their appearance in the later stages of the disease, but only when the blood of the rat was literally swarming with trypanosomes.

The close resemblance of the parasites in the gut of the louse to typical *Herpetomonas* forms described by Prowazek, Patton, etc., naturally suggested that we were perhaps dealing with a mixed infection in the rat louse—a natural infection with *Herpetomonas*, and a secondary infection with *T. lewisi*.

We made films, however, of the gut contents of about 600 lice taken from the same batch of normal rats which were afterwards used for infection, but in no instance have we been able to observe any parasites in the gut contents resembling the developmental forms of trypanosomes; only in rare instances were very small, round cells observed, which resembled gregarines. So far we have not been able to come to any definite conclusion concerning the nature of these bodies.

When lice in small numbers were placed on a clean rat, they were often rapidly eaten off by the animal and disappeared completely. On the other hand, the lice sometimes multiplied with great rapidity, so that the fur of the animal was soon swarming with them, and the animal itself soon succumbed to their injurious effects. Prowazek has already drawn attention to this difficulty.

Fresh examination.

For fresh examination, the gut contents of lice were teased out in physiological saline solution, and a coverslip preparation ringed with hard paraffin was made. In these specimens the trypanosomes could be kept alive in an actively motile condition for about thirty hours. It is a striking fact that if the lice were obtained from rats in an early stage of infection, the trypanosomes in the gut were of large size and moved sluggishly. When the rats were in a later stage of infection, the trypanosomes were small and moved at a very rapid pace, shooting across the microscopic field, so that detailed observation was almost impossible.

To obviate this difficulty a slide was first covered with a thin film of gelatine in saline solution, by which means the progressive movement of the trypanosomes was retarded. The gut contents of from three to four lice taken from a rat at a suitable stage of the infection were then taken, and specimens were made in the above described manner. The gut contents were seen to be full of parasites, some, large and moving sluggishly, others, small and resembling in their appearance living spermatozoa, possessing a small round head and a long wavy flagellum. In some instances a marked transformation was seen to take place. A large trypanosome became altered in shape; a constriction appeared at a point distant from one end about one-third of the length of the parasite. This gradually became more and more pronounced, at times disappearing, but, as a rule, in from two to four hours becoming permanent. Then the movements of the parasites quickened, and a separation into two halves, which was only connected by a thin protoplasmic strand, occurred. The parasites often remained at this stage, no further changes occurring. In only a few instances was a further development seen to take place; the anterior part of the parasite appearing to disintegrate, until, finally, only a round head with a long rapidly moving flagellum remained. In only one case was the flagellum actually seen to become detached.

No further development occurred. We have never been able to observe any process which could be satisfactorily explained as representing conjugation. Now and again, parasites apparently consisting of two changed trypanosomes, joined at one end, were observed. Although these parasites were sometimes kept under observation for twenty-four hours until all movement had ceased, in no instance did any transformation occur which could be regarded as conjugation. Tempting as it is to regard certain appearances in stained specimens as conjugation forms, nothing but observation of the whole process in fresh specimens can be regarded as conclusive.

Small, round forms, as described later in stained specimens, have frequently been seen exhibiting a circular movement.

STAINED PREPARATIONS

Changes analogous to those which we have just described can be seen in stained specimens. For the preparation of these, lice were sometimes used which had been taken from an infected rat and kept alive in a test tube for as long as seventy-two hours. After this time they usually died, unless put back on another animal.

The trypanosomes in the gut of a louse taken shortly after the height of the infection was reached usually showed quite characteristic changes in the nucleus. Whereas in a normal blood trypanosome the nucleus only contained one intranuclear centrosome (karyosome), in the majority of the louse parasites, this had divided, and the division products had moved to opposite ends of the nucleus (Figs. 1-3).

At this stage forms were found in which the nucleus had undergone complete equal division, each half containing an intranuclear centrosome surrounded by a light unstained area of karyolymph.

In a further stage (Fig. 4), one of the halves of the nucleus had moved close to the blepharoplast (extranuclear centrosome), whereas the second half seemed to lose its staining properties, taking the haematoxylin stain in a somewhat diffuse manner. The parasites were markedly increased in size. At this stage the cytoplasm appeared to become denser in that end of the parasite in which the blepharoplast was situated (Fig. 5), whereas the cytoplasm of the opposite end exhibited a somewhat ragged appearance, and seemed to have lost its well-defined outline. At a still later stage, the meshes of the 'Schaumplasma' became larger and larger and less defined, and the cytoplasm about the flagellum more and more disintegrated (Fig. 6), until, finally, only the blepharoplast end was left with a long wavy flagellum. The half of the nucleus in the flagellar end lost its staining properties to a gradually increasing extent, and sometimes the cytoplasm contained a round, sharply-defined, darkly-staining vacuole, which was probably the remains of the degenerated half of the nucleus. The other half of the nucleus underwent further changes; it increased in size and changed its position with regard to the blepharoplast, in many instances appearing behind the blepharoplast. As a rule, it contained only one intranuclear centrosome, occasionally, however, two (Fig. 7).

Judging from the number of trypanosomes in the different stages of development, it is probable that after the division of the intranuclear centrosome (karyosome), the complete division of the nucleus takes place in a comparatively short time. Forms like Figs. 1-3 and Figs. 6-8 were often seen, whereas those shown in Figs. 4-5 were comparatively rare.

Slight modifications of this process were sometimes noticed. At times the parasite did not grow to the large size seen in Figs. 4-6, whilst at other times the divided nuclei remained connected by a thin strand for a considerable time (Fig. 10).

During these changes in the nucleus and cytoplasm of the parasite, the blepharoplast did not undergo any marked transformation beyond slight enlargement. In some cases, as in Figs. 5-8, it seemed to consist of a number of round granules, but not even in the best differentiated specimens could any detailed structure be made out.

At a later stage, parasites were seen resembling spermatozoa, consisting of an oval head with a long flagellum, as well as a few forms without a flagellum. In all these stages a blepharoplast was present and could be clearly seen.

In other forms a new flagellum connected with the blepharoplast was observed. Whereas the original flagellum was thin and wavy, the new flagellum appeared very much thicker and bristle-like (Figs. 11, 12), but it seemed to become thinner as its length increased (Fig. 13). Up to this point, forms observed in stained specimens had always been controlled by examination of living parasites. Judging from the different size of the oval-shaped *Herpetomonas*-like forms of the developmental stages of *T. lewisi* (Figs. 11 and 15), one can assume that these forms increase in size.

If lice, containing a small number of these *Herpetomonas*-like forms, were taken from the rat and kept alive for a varying interval (we were not able to keep lice alive for longer than seventy-two hours), a marked multiplication of the forms took place in the gut (Fig. 18). Many of the latter, possessing only one nucleus and one blepharoplast, showed two flagella. We are inclined to regard these bi-flagellated forms as the first stage in the process of division.

The division appears to start with a broadening or rounding up of the blepharoplast (Figs. 14, 15). Afterwards, the nucleus divides and the cytoplasm of the parasite splits longitudinally into two daughter cells (Fig. 16).

Sometimes, however, before one division has come to an end, a second division has already commenced (Fig. 17). When these forms occur in large numbers, they can often be seen to be agglomerated in great clusters, with the flagellum always directed inwardly (Fig. 18). Very great variation in the shape and structure of the *Herpetomonas*-like forms was noticed (Figs. 19, 20).

In specimens stained with safranine-methylene blue, bright granules in varying numbers can very often be seen (Figs. 21, 22). Now and again the nucleus of these forms degenerates (Figs. 23, 24, 25). In Fig. 26 there is only a light area with irregular red granules in the position of the nucleus.

The stages depicted in Figs. 27 and 28 are suggestive of conjugation forms. Although analogous appearances were seen in fresh specimens and kept under observation for several hours, no further changes occurred.

Round forms. Frequently, especially in lice taken from rats at a later stage of infection, round forms, as in Figs. 32-36, were noted. They usually showed a typical oblong nucleus and a small blepharoplast, from which a long, round flagellum, which was usually wound round the parasite, originated. Whether these forms develop in the way suggested by Fig. 31, or whether they result from the coiling up of a normal trypanosome could not be determined.

LITERATURE

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DESCRIPTION OF PLATES

The figures have been drawn with an Abbé drawing apparatus, Apochromatic, 2 mm., ap. 1·4, and compensating ocular 18.

Figures 1, 2, 18, 21, 22, 26, 27 and 30 were stained with safranine-methylene-blue orange-tannin. All the others were stained by Heidenhain's method.

PLATE XIX

Figs. 1-3. Early stage, showing division of intranuclear centrosome (karyosome).

Fig. 4. Complete nuclear division. One half shows signs of degeneration.

Figs. 5, 9.—Stages showing the disintegration of part of the cytoplasm.

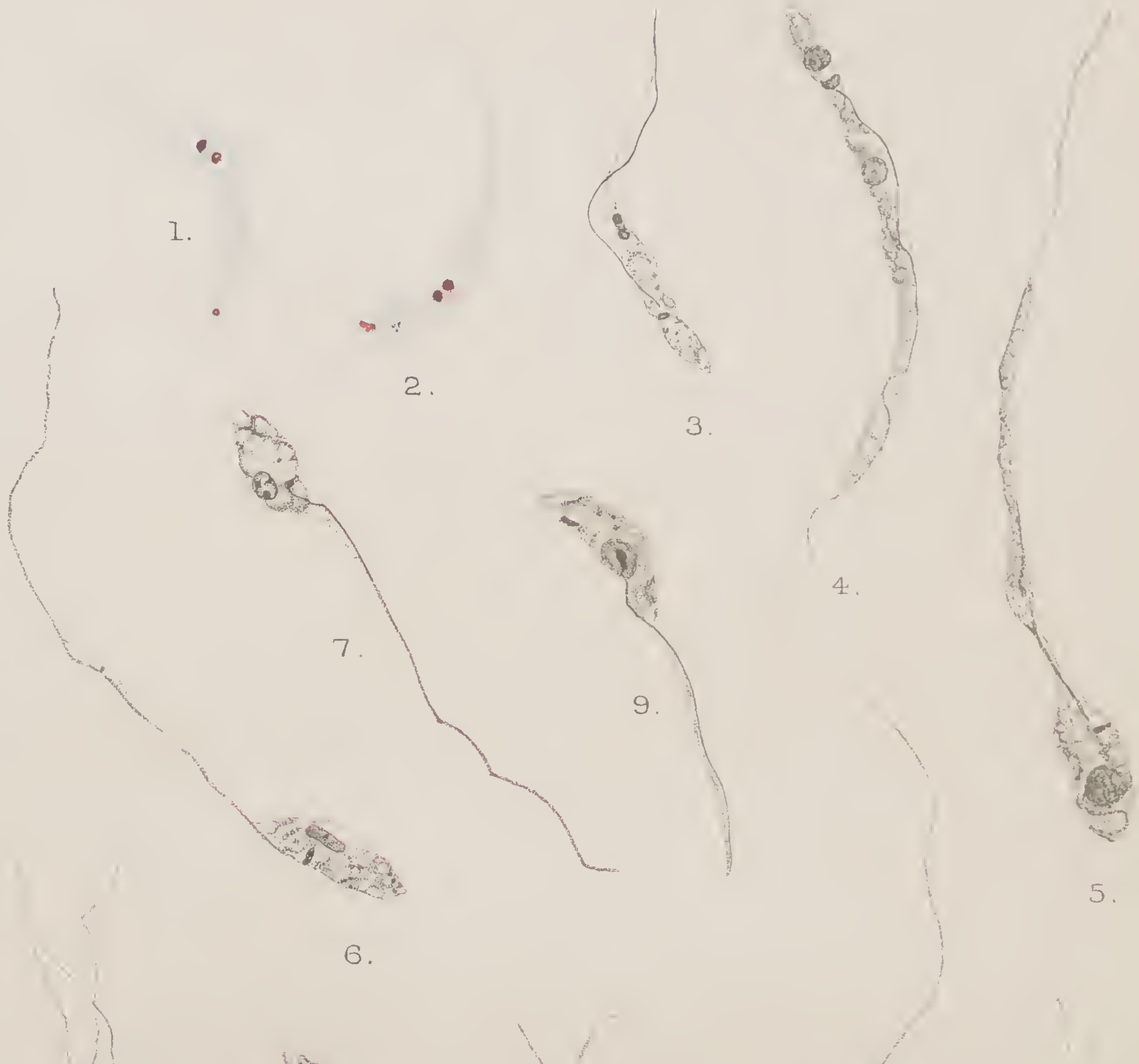
Fig. 10.—Later stage of division of the nuclei, both halves of which are still connected.

Figs. 11, 12.—Stages showing the growth of a new flagellum.

Figs. 13-15. *Herpetomonas*-like forms.

Figs. 16, 17. Division of *Herpetomonas*-like forms.

PLATE XIX



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